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MX100, a new *Escherichia coli* tester strain for use in genotoxicity studies

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The development of a new *Escherichia coli* tester strain for use in metabolic and mechanistic studies of genotoxins, strain MR2101/pKR11, has recently been reported. This strain, a derivative of the *E.coli* K12 laboratory strain AB1157, has sensitivity towards the detection of base-substitution mutagenesis, monitored by the reversion of arginine auxotrophy [*argE*3, (ochre)]. Besides arginine, MR2101/pKR11 is auxotrophic for histidine (*hisG*4), leucine (*leuB*6), proline (Δ*proA*) and threonine (*thr*-1). MX100 was developed to overcome the auxotrophy for four amino acids of MR2101/pKR11 which are non-essential for the mutagenic responsiveness of the strain. We restored the biosynthesis for these four amino acids in MR2101/pKR11, resulting in strain MX100. This strain showed an almost 2-fold increase in mutagenic activity relative to MR2101/pKR11 with a set of diagnostic mutagens (aflatoxin B1, benzo[*a*]pyrene, 4-nitroquinoline-1-oxide, 2,7-dimethylbenz[*a*]anthracene and others) and was further characterized with other types of mutagens in which it showed sensitivity towards the detection of oxidative (H_2O_2, *t*-butylhydroperoxide, cumene-hydroperoxide, KO_2) and carbonyl mutagens (methylglyoxal, malondialdehyde). As MX100 seems to have the right characteristics of a versatile genotoxicity tester strain and due to the extensive genetic and physiological knowledge of *E.coli* K12 in general and AB1157 in particular, we propose that MX100 could serve as mother strain for the development of specialized tester strains, of interest in studies of metabolism and/or mechanism of action of genotoxic carcinogens.

Introduction

We developed recently a new *Escherichia coli* K12 tester strain, MR2101/pKR11, for the study of genotoxic mechanisms and metabolism studies of chemicals (Kranendonk *et al.*, 1994a,b) in which our laboratory has been involved for a number of years (Rueff *et al.*, 1992a,b; Kranendonk *et al.*, 1993, Rodrigues *et al.*, 1993, 1994; Gaspar *et al.*, 1994). The strain is defective in the lipopolysaccharide core (LPS^d), part of the outer membrane, but contains a partial deletion of *uvrA*, a component of DNA excision repair and a newly constructed mutator plasmid (pKR11), a derivative of pKM101, carrying the SOS-inducible *mucAB* system. The genetic target by which the chemical mutagenesis is assessed consists of a point mutation (ochre) in the arginine biosynthetic operon (*argE*3) which renders the bacterium unable to grow in the absence of arginine. The mutagenicity test is based on monitoring the back mutation to arginine independence which can occur via all possible transition and transversion base-substitutions (Todd *et al.*, 1979).

We report here on the development and characterization of an improved derivative of MR2101/pKR11, strain MX100. This new strain showed increased mutagenic responses relatively to MR2101/pKR11 for a number of mutagens. The results presented here seem to characterize MX100 as a versatile genotoxicity tester strain which can be applied in a plate assay as well as in a liquid-preincubation assay, and which shows sensitivity towards a variety of different classes of genotoxins, including oxidative and carbonyl mutagens. Based on these characteristics and the extensive genetic and physiological knowledge of *E.coli* K12 in general and AB1157 in particular, we propose that MX100 could serve as the mother strain for the development of specialized tester strains, of particular interest in molecular studies of mechanisms and/or metabolism of genotoxic carcinogens.

Materials and methods

Reagents

L-Histidine (L-his), D-glucose monohydrate, crystal violet, sodium azide (NaN_3), 2-nitrofluorene (2NF), malondialdehyde (MDA), H_2O_2 and dimethyl sulphoxide (DMSO) were obtained from Merck (Darmstadt, Germany). The amino acids L-proline (L-pro), L-leucine (L-leu), L-threonine (L-thr) and L-arginine (L-arg) as well as sodium deoxycholate, ethidium bromide, 7,12-dimethylbenz[*a*]anthracene (DMBA), pyrene, cumene-hydroperoxide (CHP), *t*-butyl-hydroperoxide (tBHP), methylglyoxal (MG), quercetin (Q), mitomycin C (MMC), potassium superoxide (KO_2) and 4-nitroquinoline-1-oxide (4NQO) were purchased from Sigma (St Louis, MO, USA). Ampicillin (sodium salt) and tetracycline–HCl were from Northumbria Biologicals (Northumberland, UK). Bacto agar, bacto trypton, bacto yeast extract and bacto MacConkey agar were from Difco (Detroit, MI, USA); nutrient broth (NB) (Fluka, Buchs, Switzerland), thiamine (diphosphate form: triphosphoneurina) (Biofranco, Lisbon, Portugal) and aflatoxin B1 (AFB1) were from Aldrich (Madrid, Spain). The antibiotic kanamycin sulfate was obtained from Boehringer-Mannheim (Mannheim, Germany). Benzo[*a*]pyrene (B[*a*]P) was a gift from Pasteur Institute (Paris). Mutagens were dissolved in DMSO, except for CHP, MG, H_2O_2, KO_2 and NaN_3, which were dissolved in water.

Strains and microbiological media

All microbiological media were as described previously (Kranendonk *et al.*, 1994a). P_1^{vir} transductions and isolation of lipopolysaccharide-defective (LPS^d) mutants were performed as described previously (Kranendonk *et al.*, 1994a). The genotype and the origin of the different strains are shown in Table I.

Mutagenicity assays

Plate-incorporation assay. The mutagenicity plate-incorporation assays with MR2101/pKR11 and TA100 were performed as previously described (Kranendonk *et al.*, 1994a; Maron and Ames, 1983 respectively). MX100 was applied accordingly, without the addition of the four amino acids L-his, L-leu, L-pro and L-thr to the tester plates, unless otherwise stated

Liquid-preincubation assay. The mutagenicity assays with the mutagens H_2O_2, CHP, KO_2, tBHP, MG and MDA were performed in a liquid-preincubation assay with strains TA102 and TA104, as described by Maron and Ames (1983) with minor modifications (Abu-Shakra and Zeiger, 1990). MX100 was employed in the same way using $1–2 \times 10^8$ stationary phase cells.

At least three independent determinations were carried out for each dose level. Mutagenic activities were determined from the slope as the least-squares line of the linear portion of the dose–response curve.

Table I. Bacterial strains and plasmids

Strains	Genotype	Origin
E.coli		
AB1157	*thr-1, ara-14, leuB6, Δ(gpt-proA)62, lacY1, tsx-33, qsr⁻, supE44, galK2, λ⁻, rac⁻ hisG4, rfbD1, mgl-51, rpsL31, kdgK51, xyL-5, mtl-1, argE3, thi-1*	B.Bachmann
AB1886	AB1157, *uvrA6*	B.Bachmann
MR2100	AB1886, *galE*	M Kranendonk
MR2101	MR2100, LPS defective	M.Kranendonk
MM294	*supE44, λ⁻, endA1, thi-1, hsdR17*	B.Bachmann
FP100	MR2100, *his*$^+$	this paper (P1[MM294]×MR2100 to His$^+$)
FP200	FP100, *pro*$^+$	this paper (P1[MM294]×FP100 to Pro$^+$)
FP300	FP200, *leu*$^+$	this paper (P1[MM294]×FP200 to Leu$^+$)
FP400	FP300, *thr*$^+$	this paper (P1[MM294]×FP300 to Thr$^+$)
FP401	FP400, LPS defective	this paper (C21^r selection on FP400)
MX100	FP401/pKR11	this paper
S.typhimurium		
TA100	*hisG46, Δ(gal-uvrB), rfa/* pKM101	B N.Ames
TA102	*hisΔ(G)8476, rfa/ pAQ1/* pKM101	B.N.Ames
TA104	hisG428, *Δ(gal-uvrB), rfa/* pKM101	B.N.Ames
Plasmids	**Relevant genetic markers**	**Origin**
pKM101	*mucAB*$^+$, Amp^r	B.N.Ames
pAQ1	*hisG428*, Tet^r	B.N.Ames
pKR11	*mucAB*$^+$, Kan^r	M.Kranendonk

Results

Development of strain MX100

The *E.coli* K12 strain MR2101/pKR11, a derivative of the laboratory strain AB1157 (see Table I), seems to have the necessary characteristics of a genotoxicity tester strain, developed especially for mechanistic and metabolic studies of mutagens (Kranendonk *et al.*, 1994a,b). Besides the arginine auxotrophy, due to the *argE*3 mutation which constitutes the genetic target, it is auxotrophic for histidine [*hisG*4 (ochre)], leucine (*leuB*6), proline (Δ*proA*) and threonine [*thr*-1 (amber)], which is the origin of the impractical addition (in excess) of these four amino acids to the tester plates. We decided to restore the biosynthesis capacity of these four amino acids by four subsequent P_1^{vir} transductions using an appropriate donor strain to remedy the four afflicted genes in MR2101/pKR11. The arginine reversion by which mutagenicity is assessed can occur via (i) structural reversion of the *argE*3 (ochre) mutation to a sense codon or conversion to an amber codon which can be suppressed by the *supE*44 mutation; (ii) ochre suppressor formation *de novo*; or (iii) suppressor conversion of *supE*44 to *supB* (Todd *et al.*, 1979). These mutations cover all possible transitions and transversion base-substitutions. The donor strain [MM249 (*supE*44); see Table I] was chosen carefully so that no interfering mutations would be introduced. In this way all the elements of the genetic target were maintained and the prototrophy for the four amino acids was in principle restored by the introduction of the wild-type form of the four afflicted genes but not by the introduction of a new suppressor mutation. The only way proline prototrophy could be restored was by introduction of the wild-type gene of *proA* as this gene is deleted in MR2101/pKR11. The four P1[MM294] transductions were carried out with the LPS$^+$ predecessor of strain MR2101 (MR2100; see Table I) as LPS^d bacteria are (partially) resistant to P_1 infection (Austin *et al.*, 1990; Parker *et al.*, 1992). A stable LPS^d mutant was isolated (FP401) with high permeability towards bulky compounds, which was similar to the one found for MR2101 (data not shown). Subsequently our mutator plasmid pKR11 (Kranendonk *et al.*, 1994a) carrying the *mucAB* operon was introduced, resulting in strain MX100.

Mutagenicity testing with MX100

Before employing MX100 in mutagenicity assays, monitoring the arginine reversion, the optimum trace amount of arginine was verified, as determined before for MR2101/pKR11, to allow a limiting number of DNA replications of the tester bacteria in the presence of the test compound. This growth leads to a more accessible DNA target and allows the processing of the DNA lesion into a mutation (genetic fixation) (Mohn *et al.*, 1984). The optimal L-arg concentration (1 μg/ml) was found to be equal to that found for MR2101/pKR11 (Kranendonk *et al.*, 1994a). In these optimal conditions 205 revertants were picked up arbitrarily from different tester plates induced by different test compounds (see below) and checked for their true Arg$^+$ character. All 205 revertants maintained their Arg$^+$ character on subculture. A set of diagnostic compounds [as used by Kranendonk *et al.* (1994a)] was tested with MX100 and compared with MR2101/pKR11 (see Table II). Almost all mutagens showed an increase in mutagenic activity (in revertants/nmol) with MX100 relative to MR2101/pKR11, except for sodium azide [false positive of TA100 (McCann *et al.*, 1975)] and pyrene, which both remained negative. On average, the mutagenic response of MX100 was increased almost 2-fold relative to MR2101/pKR11 (1.70 ± 0.01, $r = 0.999$) for the compounds tested (see Figure 1). As the LPS^d mutations of MX100 and MR2101/pKR11 were isolated independently, the increase in responsiveness could be due to differences in the ease of penetration of the test compounds. This hypothesis is excluded as both strains showed similar permeabilities of the LPS core. The composition of the tester plate of the two *E.coli* strains differ as the four amino acids could be omitted from the MX100 tester plates. To verify whether the four amino acids could influence the magnitude of the mutagenic response, the mutagenic activity of 4NQO was determined in MR2101/pKR11 and MX100 with and without the four amino acids present in the tester

Table II. Average results of the compounds tested with MR2101/pKR11 and MX100

Compound	nmol/plate	MR2101/pKR11		MX100	
		Rev/plate	SD	Rev/plate	SD
–S9					
NaN_3	0.0	79.7	9.8	65.7	2.1
	6.2	81.0	6.2	60.7	8.0
	12.3	79.7	9.5	48.7	5.1
	18.5	86.0	14.9	58.3	6.1
	24.6	81.7	16.9	52.7	4.2
	30.8	88.3	13.2	59.7	8.7
2NF	0.0	74.8	12.7	93.3	3.2
	11.8	–	–	161.0	8.2
	23.7	115.3	14.1	202.7	12.4
	35.5	–	–	248.7	6.7
	47.3	143.5	20.2	270.0	25.4
	59.2	–	–	302.7	11.4
	71.0	178.0	51.8	244.3	8.4
	94.7	163.5	47.2	–	–
	118.4	166.0	65.9	–	–
Quercetin	0.0	79 7	9.8	65.0	5.3
	66.2	213.0	44.0	179.0	44.3
	132.3	311.7	88.6	275.7	95.6
	198.5	350 0	85.8	320.7	90.4
	264.6	448.7	113.5	370.0	103.4
	330.8	391.0	101.9	387.7	115.7
4NQO	0.00	84 5	12.6	70.5	13.8
	1.05	143.3	25.1	281.5	98.2
	2.10	308.3	66.2	590.8	127.7
	3.16	505.5	85.0	980.2	91.2
	4.21	860.0	131.2	1416.2	129.2
	5.26	1256.0	265.5	2020.8	285.9
	6.31	1628.0	483.4	2565.2	388.9
	7.36	2109.7	729.9	3017.3	297.3
+S9					
Pyrene	0.0	79.3	12.0	92.3	13.6
	197.8	107.7	33.6	81.0	8.5
	395.5	98.3	24.7	81.3	12.7
	593.3	94.0	18.1	84.7	9.0
	791.1	99.0	19.5	78.7	20.7
	988.8	92.5	21.6	75.0	13.0
DMBA	0.0	81.5	28.8	76.3	12.2
	15.6	113.8	20.8	141.7	43.7
	31.2	174.3	29.8	320.7	173.4
	46.8	212.3	55.2	341.3	60.9
	62.4	221.0	33.0	399.0	89.2
	78.0	220.3	68.9	366.7	53.9
Quercetin	0.0	87.8	21.8	88.0	2.0
	66.2	403.8	93.9	316.0	31.2
	132.3	466.5	31.7	456.0	48.8
	198.5	503.0	64.3	531.3	68.3
	264.6	585.3	70.2	631.7	89.5
	330.8	580.0	64.6	615.7	70.5
B[*a*]P	0.0	92.3	29.5	77.7	8.8
	4.0	227.7	92.1	197.0	34.8
	7.9	389.3	116.8	377.0	52.9
	11.9	540.0	162.7	466.3	75.8
	15.9	682.7	83.3	599.0	147.0
	19.8	722.7	134.0	603.0	98.9
AFB1	0.000	89.7	15.8	88.3	1.5
	0.128	477.0	66.5	840.7	1.2
	0.256	855.0	38.2	1390.3	45.5
	0.384	904.7	38.8	1488.3	154.0
	0.512	1096.3	30.1	1646.0	129.4
	0.640	939.3	104.3	1641.3	151.3

Results are shown as means representing at least three independent determinations for each dose-level ± SD.

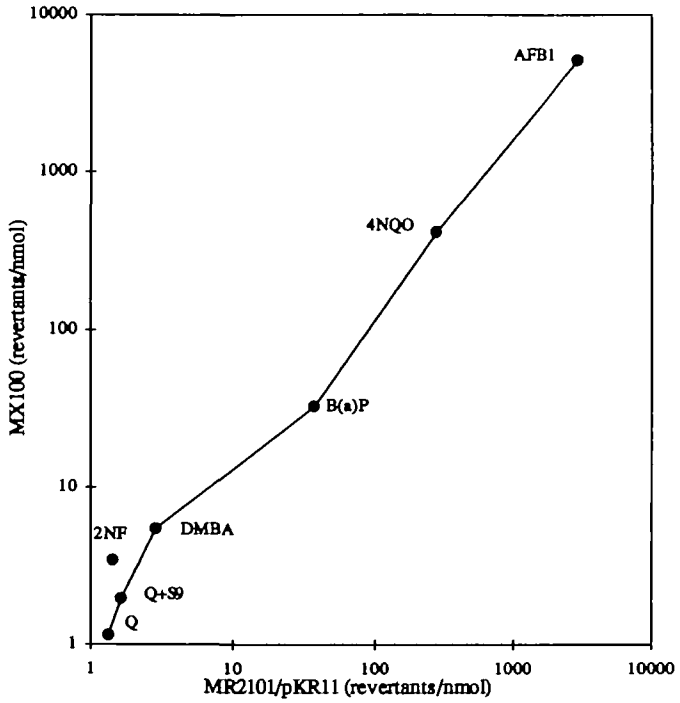

Fig. 1. Correlation of specific mutagenic activities of strain MR2101/pKR11 against MX100. Mutagenic activities were determined as the least-squares line of the linear portion of the dose–response curve.

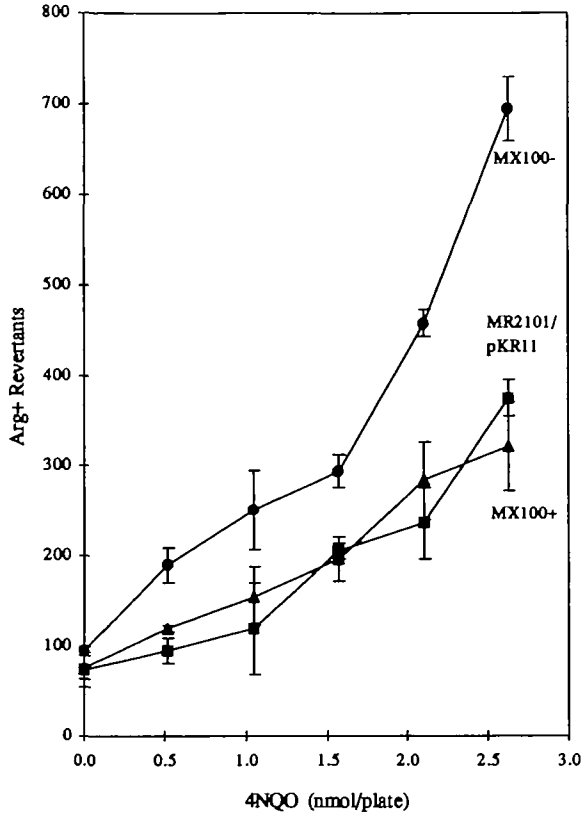

Fig. 2. Mutagenic activity of 4NQO in strains MR2101/pKR11 and MX100 with and without the amino acids L-his, L-leu, L-pro and L-thr as applied for MR2101/pKR11 (100 μg/ml, final plate concentration). $MX100^-$: without the amino acids; $MX100^+$: with the amino acids.

plates, as for MR2101/pKR11 (see Figure 2). The mutagenic response of MX100 for 4NQO in the presence of the amino acids regressed to the level of that found for MR2101/pKR11. We have found this effect for other mutagens (data not shown). The presence of the amino acids seem to diminish the mutagenic responsiveness of MX100 and it seems that, because they could be omitted from the minimal tester medium, the mutagenic response of MX100 is improved.

Further characterization of MX100

We extended the number of test compounds to further characterize MX100 and to verify the sensitivity of the arginine target towards other classes of mutagens. Our laboratory is involved in studies of oxidative mutagenesis (Almeida *et al.*, 1992; Cristóvão *et al.*, 1992; Rueff *et al.*, 1992a, 1993; Gaspar *et al.*, 1994). MX100 was therefore tested with H_2O_2, CHP, tBHP and KO_2, to determine the usefulness of MX100 for such studies, and was compared with strain TA102, which together with strain TA104 was originally developed for the detection of oxidative mutagens (Levin *et al.*, 1982) (see Table III). The mutagenic responses of these compounds were determined in a liquid-preincubation assay in which oxidative mutagens seem to be more readily detected (Abu-Shakra and Zeiger, 1990). The results show that MX100 is sensitive towards the detection of this class of mutagen, and can be employed effectively in a liquid-preincubation assay. Moreover, MX100 has a stable low background reversion (in spontaneous revertants) in contrast to the two *Salmonella* strains, which forms a major practical drawback, especially for TA102 (Jung *et al.*, 1992). The genetic target in TA102 and TA104 is constituted by the *hisG*428 (ochre) mutation, which is located plasmid-borne in TA102 and genomically in TA104. Furthermore, TA104 is hampered in DNA excision repair (*uvrB*), in contrast to TA102 (uvr^+). The His reversion of these strains seems to occur preferentially at the *hisG* site rather than ochre suppressor mutations outside the *his* operon (Levin *et al.*, 1982). The target is therefore constituted only by A:T base pairs which are equally present in the *argE*3 (ochre) mutation of MX100, forming indicators for the observed sensitivity of MX100 for oxidative mutagens. This is in concordance with the sensitivity of the Trp^+ reversion in the *E.coli* tester strain WP2 containing the *trp*E (ochre) mutation towards the detection of oxidative mutagens (Wilcox *et al.*, 1990). MX100 is more closely related to TA104 than to TA102, as both are excision repair deficient and contain both genomic (ochre) targets (see Table I). TA104 has been shown to be sensitive towards the detection of carbonyl mutagens which apparently have an oxidative component in their mutagenic action (Marnett *et al.*, 1985; Ueno *et al.*, 1991). MX100 was therefore subsequently tested with two carbonyl mutagens [methylglyoxal (MG) and malondialdehyde (MDA)], in comparison with TA104 (see Table III). TA102 and TA104 differ in their sensitivity towards the detection of DNA cross-linking agents which require an active excision repair for mutagenic activity (Levin *et al.*, 1982). This is confirmed by the results obtained with the DNA cross-linker mitomycin C (MMC) in the strains TA102, TA104 and MX100 (see Table III). The results obtained with the last three compounds seem to confirm the structural similarities between MX100 and TA104 and therefore the abilities of MX100 to detect oxidative mutagens.

Discussion

We have described here the development and characterization of MX100, an improved derivative of MR2101/pKR11. MX100

Table III. Average results of the compounds tested with MX100, TA102 and TA104

Compound	μmol/plate	MX100		TA102	
		Rev/plate	SD	Rev/plate	SD
H_2O_2	0.0	66.7	5.7	325.0	28.1
	1.2	–	–	534.0	66.8
	2.4	–	–	669.0	150.7
	3.5	–	–	1064.7	163.1
	4.7	–	–	1100.0	204.1
	5.9	222.7	24.2	1183.3	89.5
	11.8	428.0	60.0	–	–
	17.6	811.3	74.1	–	–
	23.5	1065.3	139.6	–	–
	29.4	1232.3	248.3	–	–
tBHP	0.000	83.3	5.0	392.3	45.5
	0.111	149.3	9.1	478.7	34.5
	0.222	219.7	23.6	920.0	51.7
	0.333	481.0	17.7	1033.7	22.6
	0.444	759.3	99.9	1133.7	139.7
	0.555	951.7	92.5	1148.0	61.6
CHP	0.000	56.7	1.5	398.7	29.4
	0.197	396.0	27.1	1240.3	90.3
	0.329	–	–	1502.7	181.8
	0.394	595.3	90.8	–	–
	0.460	–	–	1796.0	218.5
	0.591	843.0	26.7	1832.0	123.0
	0 788	920.0	93.0	–	–
	0.986	1043.7	98.1	–	–
KO_2	0.0	74.3	14.5	331.0	26.3
	2.8	103.3	15.0	370.7	4.9
	5.6	121.3	18.2	417.3	62.8
	8.4	128.3	18.8	431.3	19.6
	11.3	134.0	6.6	495.0	16.7
	14.1	160.7	23.1	543.0	73.0

Compound	μmol/plate	MX100		TA104	
		Rev/plate	SD	Rev/plate	SD
MDA	0.0	67.0	25.1	651.0	33.5
	15.2	167.7	26.3	851.0	35.7
	30.5	269.3	14.6	865.0	4.0
	45.7	267.0	7.0	920.0	192.5
	60.9	320.7	26.6	447.3	56.9
	76.1	421.7	17.2	151.7	41.0
MG	0.000	75.0	2.7	492.0	16.5
	0.058	121.3	2.9	915.3	145.0
	0.116	252.3	15.0	1148.0	10.5
	0.174	474.7	9.0	1838.7	62.3
	0.232	884.0	20.8	2852.0	273.9
	0.291	1523.3	35.5	–	–
	0.349	1824.7	126.6	–	–

Compound	nmol/plate	MX100		TA102		TA104	
		Rev/plate	SD	Rev/plate	SD	Rev/plate	SD
MMC	0.000	63.3	2.3	206.7	14.5	512.0	35.5
	0.060	73.3	7.6	744.3	85.9	440.7	31.6
	0.120	67.7	0.6	1021.7	57.5	391.3	15.0
	0.179	80.0	13.5	1261.3	110.4	369.0	19.9
	0.239	91.7	3.5	1471.3	98.7	324.0	11.5
	0.359	97.7	8.6	1980.0	142.7	262.7	31.1

Results are shown as means representing at least three independent determinations for each dose-level ± SD.

was developed to overcome the auxotrophy of MR2101/pKR11 for four amino acids which are non-essential for the mutagenic responsiveness of the strain. The biosynthesis of these amino acids was restored in such a way that in principle no undesired genetic alterations were introduced which could interfere with the different ways of the arginine back mutation, as the *hisG*4, *leuB*6 and *thr*-1 mutations can be suppressed by specific suppressor mutations (Todd *et al.*, 1979). Such mutations could

Table IV. Comparison of mutagenic activity of various mutagens in strain MX100 versus the routinely used strains *E.coli* WP2 and *Salmonella* TA100 and TA102

Compound	WP2	WP2-uvrA	WP2-pKM101	WP2-uvrA pKM101	TA102	TA100	MX100	Ref.[b]
2NF		+[a]				+	+	1, 2, 3
4NQO	+	+	+	+		+	+	2, 3, 4
AFB1	–	–	–			+	+	2, 3, 4
B[*a*]P	–	+	+	+		+	+	2, 3, 4
CHP			+	+	+		+	2, 3, 5
DMBA	–	–	+			+	+	2, 3, 4
H2O2			+	+	+		+	2, 3, 5
NaN3	–	–				+	–	1, 2, 3
MMC	+	–	+	–	–		–	1, 2, 3, 5

[a]Mutagenicity: (+), mutagenic; (–), non-mutagenic.
[b]References: 1, Brusick *et al.* (1980); 2, Kranendonk *et al.* (1994); 3, this paper; 4, Dyrby and Ingvardsen (1983); 5, Wilcox *et al.* (1990).

have nullified part of the (multiple) arginine target which would have led to a decrease in mutagenic responsiveness. As the opposite was found, it is concluded that the restored biosynthesis pathways in MX100 are due to the substitution of the afflicted genes.

MX100 showed an almost 2-fold increase in specific mutagenic activity with a set of diagnostic compounds relative to MR2101/pKR11. The compositions of the tester plate of the two *E.coli* strains differ as the four amino acids could be omitted from the MX100 tester plates. We showed that it is probably because of this omission that MX100 seems to be improved. It is tempting to speculate that amino acid starvation of MX100 relative to MR2101/pKR11 could, in a way similar to glucose starvation (Kopsidas and MacPhee, 1993), diminish the efficiency of error-free repair with a parallel augmented error-prone repair, explaining the observed increase in mutagenic activity for MX100.

We showed here that the back mutation can also be induced by known oxidative and carbonyl mutagens. The results obtained for MX100 with the wide spectrum of different mutagens reflects the different ways by which the arginine auxotrophy can revert, covering all possible transitions and transversions. This indicates the versatility of the arginine back mutation as the genetic target in MX100 and seems to characterize MX100 as a tester strain which can be of interest in studies of the mode of action of mutagens with multiple genotoxic effects. Table IV shows a comparison of mutagenicity results obtained in MX100 versus routinely used tester strains, namely *E.coli* WP2 and *Salmonella* strains, demonstrating that the responses of MX100 match the ones found in the other tester strains. MX100 could, however, have some advantages over the routinely used tester strains in mechanistic and metabolic studies of genotoxins because MX100 is derived from the *E.coli* K12 laboratory strain AB1157, a strain which is genetically very well characterized for DNA repair, especially for SOS mutagenesis (Langer *et al.*, 1981, 1985; Sedgwick and Goodwin, 1985; Donnelly and Walker 1989, 1992; Sedgwick *et al.*, 1991; Sommer *et al.*, 1993). Moreover, AB1157 has been shown to be one of the more mutable *E.coli* strains (Sedgwick *et al.*, 1991).

The loci of genes whose products could be involved in metabolism of genotoxins are in general best characterized in *E.coli* K12 relative to other prokaryotes (Mohn *et al.*, 1984; Kranendonk *et al.*, 1994a). This can be beneficial in studies of the involvement of bacterial metabolism in the mutagenic effect of genotoxins. *Escherichia coli* is an attractive organism for studies of the involvement of the different repair mechanisms in mutagenesis, as these mechanisms occur in *E.coli* via basic molecular processes which have their counterparts in higher, though much more complex, cells (Walker, 1985; Sancar and Sancar, 1988), such as recombinational repair (Kowalczykowski *et al.*, 1994), excision repair (Barnes *et al.*, 1993) and mismatch repair (Lindahl, 1994). This last mentioned repair has been in focus recently, demonstrating the importance of mutagenesis studies in *E.coli* and the application of strain AB1157, due to the characterization of a locus in the human genome, coding for human MSH2, a homologue of *E.coli* Mut S and yeast MSH, the mutation of which is associated with hereditary non-polyposis colon cancer (HNPCC) (Fishel *et al.*, 1993). The expression of human MSH2 in *E.coli* (AB1157) demonstrated a physiological activity in *E.coli* mismatch repair (Fishel *et al.*, 1993).

It is due to the above described characteristics of *E.coli* in general and AB1157 and MX100 in particular that we propose here that MX100 could serve as the mother strain for the development of specialized tester strains, of interest in studies of metabolism and/or the mechanism of action of genotoxic carcinogens. These can be: (i) introduction of specific genetic targets (either genomic or plasmid-borne) by which the preferences of mutations induced by specific genotoxins can be studied; (ii) inactivation or overexpression of genes whose products are involved in specific DNA repair mechanisms, by which the involvement of specific DNA repair processes in the induction of specific mutations can be determined; (iii) inactivation or overexpression of specific bacterial genes whose products could be involved in the bio(in)activation of pregenotoxins; and (iv) expression of specific mammalian metabolizing enzymes. The latter is especially of interest because in the last few years much effort has been put into the unravelling of human metabolic pathways, involved in bio(in)-activation of genotoxins. One of the more promising approaches is the direct introduction of genes/cDNAs of mammalian metabolizing enzymes in the genotoxicity assay target cell (Gonzalez *et al.*, 1991; Rodrigues *et al.*, 1993). *Escherichia coli* seems to be particularly appropriate for this application (Kranendonk *et al.*, 1994b). At the moment, MX100 is being applied in all four mentioned areas.

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